FEBS Letters 368 (1995) 220–224 FEBS 15720

Direct electrochemistry and EPR spectroscopy of spinach ferredoxin mutants with modified electron transfer properties

Alessandro Aliverti[a], Wilfred R. Hagen[b,*], Giuliana Zanetti[a]

[a]*Dipartimento di Fisiologia e Biochimica Generali, Università degli Studi di Milano, Milan, Italy*
[b]*Department of Biochemistry, Wageningen Agricultural University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands*

Received 4 May 1995; revised version received 31 May 1995

Abstract Mutations of the conserved residue Glu-92 to lysine, glutamine, and alanine have been performed in the recombinant ferredoxin I of spinach leaves. The purified ferredoxin mutants were found twice as active with respect to wild-type protein in the NADPH-cytochrome *c* reductase reaction catalyzed by ferredoxin-NADP$^+$ reductase in the presence of ferredoxin. Cyclic voltammetry and EPR measurements showed that the mutations cause a change in the [2Fe–2S] cluster geometry, whose redox potential becomes approximately 80 mV less negative. These data point to a role of the Glu-92 side-chain in determining the low redox potential typical of the [2Fe–2S] cluster of chloroplast and cyanobacterial ferredoxins. Also a ferredoxin/ferredoxin-NADP$^+$ reductase chimeric protein obtained by gene fusion was overproduced in *Escherichia coli* and purified. Fusion of the ferredoxin with its reductase causes only minor effects to the iron–sulfur cluster, as judged by cyclic voltammetry and EPR measurements.

Key words: Ferredoxin I; Iron–sulfur cluster; Site-directed mutagenesis; Redox potential; Cyclic voltammetry; EPR

1. Introduction

In aerobic photosynthesis, NADP$^+$ is the terminal acceptor of reducing equivalents from photosystem I. Electrons are transferred to NADP$^+$ from the [2Fe–2S]$^{1+}$ cluster of ferredoxin through the catalytic action of ferredoxin-NADP$^+$ reductase (FNR). The molecular details of the interaction between the two proteins, the ferredoxin and the reductase, are not established; however, a crucial role has been putatively ascribed to surface electrostatic interactions between the negatively charged ferredoxin and positive charges on the reductase [1–3]. More recently, a specific glutamate residue of ferredoxin has been proposed to be essential in the electron-transfer process on the basis of site-directed mutagenesis studies of the Anabaena protein [4]. This Glu-94 is on the protein surface [5]; furthermore, it has been shown from the crystal structure of the Anabaena ferredoxin at 1.7 Å resolution that its side-chain carboxylate forms a hydrogen bond to Ser-47, which is part of the [2Fe–2S] binding loop [6]. Upon mutating this glutamate into a positively charged lysine (E94K) the electron transfer rate from ferredoxin to reductase dropped by four orders of magnitude, as measured by laser flash photolysis [4]. This effect was ascribed entirely to a modified surface interaction since no significant change was observed in the redox potential of the iron–sulfur cluster or in the optical properties of the protein.

Here we report on a similar study involving the ferredoxin I (FdI) and FNR from spinach leaves. The residue Glu-94 of the *Anabaena* ferredoxin corresponds to Glu-92 in the spinach FdI. In contrast to the results previously reported for the *Anabaena* ferredoxin, we found that the Glu-92 mutation in spinach FdI does not impair electron transfer and does have a drastic effect on the iron–sulfur cluster redox potential. Also, the EPR spectrum of the [2Fe–2S]$^{1+}$ cluster is changed.

2. Materials and methods

Recombinant spinach FNR and wild-type FdI were purified from *Escherichia coli* as outlined in [7] and in [8], respectively. Mutant FdI forms (namely Fd-E92Q, Fd-E92A, and Fd-E92K) were obtained by oligonucleotide-directed mutagenesis, and were purified to homogeneity by the same procedure used for wild-type FdI. Details about mutagenesis will be reported elsewhere. The chimeric fusion protein between FdI and FNR (Fd-FNR) was obtained by expressing in *E. coli* a gene construct where the 3′ end of the mature FdI coding sequence was fused to the 5′ end of the mature FNR coding sequence (unpublished results). The protein was isolated by a procedure similar to that used for FdI purification. Absorption spectra were recorded with a Hewlett-Packard diode-array 8452A spectrophotometer. Ferredoxin-dependent cytochrome *c* reductase activity of FNR was measured at 25°C in 0.1 M Tris-HCl, pH 8.2, in the presence of 0.1 mM NADPH, 7 μM FdI form and cytochrome *c* (1 mg/ml). An NADPH-regenerating system comprising 2.5 mM glucose-6-phosphate and excess glucose-6-phosphate dehydrogenase was also present. Cytochrome *c* reduction was monitored spectrophotometrically at 50 nm. Cyclic voltammetry in 10 μl volumes was done at 20°C in the previously described three-electrode microcell [9]. The cell was driven by a BAS CV-27 analogue potentiostat (BioAnalytical Systems, IN, USA) connected to an *x–y*-recorder (Kipp&Zonen, The Netherlands) and an accurate voltmeter (Fluke, NL). The working electrode was glassy carbon, the counter electrode was platinum wire, and the reference was a saturated calomel electrode (+247.7 mV vs. NHE at 20°C). All reported potentials have been recalculated with respect to the NHE. The glassy carbon surface was activated by oxidation in 65% HNO_3, neutralization, mild polishing with 0.6 μm particles [9] followed by heating to redness over a methane flame to obtain a hydrophilic surface [10]. The final solution contained ca. 2 mg/ml protein in buffer plus 2 mM of the promoter neomycin sulfate. The electrochemical response of the ferredoxin was not indefinitely stable; however, working with rigorously and freshly cleaned electrodes allowed for the development of a stable response after 4–6 cyclic scans at a potential scan rate of $v = 20$ mV/s, followed by a measuring period of 10–15 min. After this period the response would tend to deteriorate as evidenced by a slowly increasing cathodic to anodic peak potential difference and a slowly decreasing peak current. EPR spectroscopy was done on a Bruker 200 D spectrometer equipped with a home-built helium-flow cryostat and with other peripheral equipment and data acquisition facilities as previously described [11]. Quantitative reduction of ferredoxin samples was achieved by anaero-

*Corresponding author. Fax: (31) (8370) 84-801.
E-mail: fred.hagen@epr.bc.wau.nl

Abbreviations: FNR, ferredoxin-NADP$^+$ reductase; FdI, ferredoxin I; Fd-FNR, fusion between FdI and FNR; NHE, normal hydrogen electrode.

SSDI 0014-5793(95)00648-6

bic dilution (1:1) with buffered sodium dithionite (final concentration 5 mM).

3. Results

3.1. Isolation and activity of Glu-92 mutants of FdI

With the aim of clarifying the role of the conserved residue Glu-92 of spinach FdI, three mutant protein forms have been obtained by site-directed mutagenesis, overproduced in *E. coli* and purified (manuscript in preparation). The side chain of residue 92 has been changed to lysine, glutamine and alanine. All FdI mutants had absorbance spectra not significantly different from that of the wild-type protein (not shown), as reported also for the corresponding mutants of the *Anabaena* ferredoxin [4]. A convenient and rapid method to test the functionality of the mutant FdI forms is to measure the ability of the mutant proteins to support the NADPH-cytochrome *c* reductase activity of FNR, which is strictly dependent on the presence of ferredoxin in the assay medium. In this reaction the electrons flow in the direction opposite to the physiological one. Surprisingly, all the FdI mutants showed enhanced activity in comparison to the wild-type protein. With 7 μM FdI form present in the assay the wild-type protein afforded an activity of 3540 U/FAD, while the mutants gave approximately two-fold higher activities, namely, E92K, 242%; E92Q, 190%; and E92A, 219%. These data suggested that the mutations could affect the redox potential of the [2Fe–2S] cluster of ferredoxin.

3.2. Electrochemistry

The direct electrochemistry of spinach ferredoxin has been previously described as a quasi-reversible response on glassy carbon [10]. Wild-type spinach ferredoxin is a mixture of two proteins with strong sequence homology. Here, we use a recombinant protein, i.e. the spinach FdI overproduced in *E. coli* [8]. The response of this homogeneous preparation on glassy carbon is indistinguishable from that previously observed with the natural mixture (see Fig. 1, Table 1, [10]). Therefore, below we refer to the homogeneous FdI as wild-type. The response is essentially diffusion controlled, or quasi-reversible [12,13] at low potential scan rates: the ratio of cathodic and anodic peak current is near unity (Fig. 1) and the peak current increases linearly with the square root of the potential scan rate v at least up to v = 100 mV/s (not shown). However, with increasing scan rates the electron transfer between protein and solid electrode becomes kinetically limiting as evidenced from an increasing separation between the potentials (ΔE) corresponding to cathodic and anodic peak current.

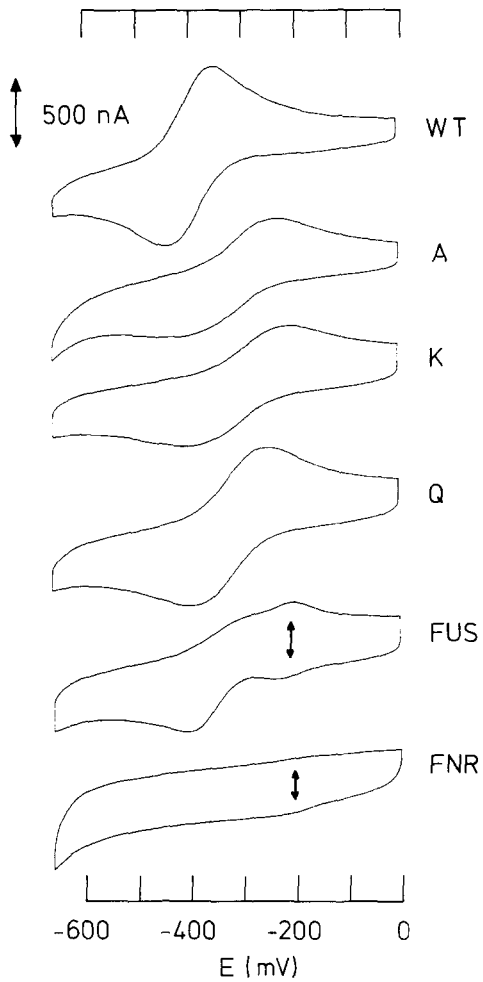

Fig. 1. Cyclic voltammograms of spinach ferredoxins and fusion protein Fd-FNR: wild-type FdI (trace WT, 240 μM), E92K mutant (trace K, 179 μM), E92A mutant (trace A, 197 μM), E92Q mutant (trace Q, 178 μM), fusion protein Fd-FNR (trace FUS, 62 μM), and reductase (trace FNR, 120 μM). All proteins were in 75 mM Tris-HCl, pH 7.4 and 2 mM neomycin. The potential scan rate v = 5 mV/s and the temperature was 20°C. Responses of free FAD are indicated with an arrow.

Table 1
Redox potentials and heterogeneous electron transfer rate constants of spinach ferredoxin mutants as determined by direct electrochemistry on glassy carbon

FdI form	$E_{m,7.4}$[a] (mV)	ΔE[b] (mV)	$k^0 \times 10^{-4}$[c] (cm/s)
Wild-type	−401	75	14.0
E92K	−308	148	2.0
E92Q	−328	119	2.9
E92A	−323	132	2.3
FD-FNR	≈−352	≈85	7.8

[a] Conditions were as in Fig. 1.
[b] Separation of potentials corresponding to cathodic and anodic peak currents determined using a potential scan rate of 5 mV/s.
[c] This rate constant was determined from ΔE, assuming a diffusion constant $D_{ox} = D_{red} \approx 1 \times 10^{-6}$ cm²/s [12].

Mutation of the negatively charged Glu-92 into a positively charged lysine has two effects on the electrochemical response. The $[2Fe–2S]^{(2+;1+)}$ redox potential increases by 93 mV from −401 to −308 mV. Secondly, there is a substantial decrease in the rate of electron transfer as evidenced by an increase in the ΔE (Fig. 1 and Table 1). The peak current increases linearly with $v^{0.5}$ up to v = 100 mV/s, therefore, the response is still quasi-reversible. The effects are not simply the result of the positive versus negative charge substitution. When the electrochemistry is repeated for a FdI form where a negative-to-neutral charge change was obtained by replacing Glu-92 with glutamine (E92Q), a comparable redox potential shift of +73 mV is found. However, the decrease in electron-transfer rate is now less pronounced than that for the E92K mutant. When the side chain of the glutamate is removed by replacing it with an alan-

ine residue (E92A), again a comparable shift in the redox potential of +78 mV is found. For this mutant the electron-transfer rate is again decreased and comparable to that of the Fd-E92K.

The chimeric protein Fd-FNR, the expression product of a fusion between the genes of FdI and its reductase, has a molecular mass of 45.5 kDa (10.5 + 35 kDa). Also this protein affords quasi-reversible cyclic voltammetry, which is remarkable because enzymes usually do not give a direct response on solid electrodes. In fact, the reductase by itself does not give a response except for a weak signal from FAD dissociated from the protein and adsorbed onto the electrode (Fig. 1). This is not an uncommon observation for flavoproteins [14]. The adsorption is borne out by the observation of a $\Delta E \approx 0$ mV. In this respect the fusion protein Fd-FNR appears to be somewhat less stable than the reductase as it exhibits a relatively stronger free FAD response. Analysis of the direct electrochemical response from the chimeric protein is hampered by the interfering flavin signal. This is especially the case at increased scan rates, because the peak current from the absorbed FAD signal increases linearly with v while the diffusion-controlled response of the protein increases linearly with $v^{0.5}$. Therefore, we have only been able to confirm linearity with $v^{0.5}$ up to $v \approx 20$ mV/s. The determination of the redox potential E_m and the potential peak separation ΔE (Table 1) have an estimated added uncertainty of ± 10 mV compared to the ferredoxin values. We have previously shown that spinach ferredoxin in the presence of its reductase and $NADP^+$ exhibits a catalytic wave in electrochemistry reflecting NADPH production [10]. A very similar wave is observed with the chimeric protein Fd-FNR in the presence of $NADP^+$ (not shown). The observed redox potential for the fusion protein is −352 mV. It is not obvious what redox process is measured in this response because the protein contains a [2Fe–2S] cluster and an FAD. We tentatively ascribe the response to the iron–sulfur cluster because: (1) the reductase itself is electrochemically inactive; (2) the amplitude of the peak current is equal to that of FdI alone when the concentrations are normalized; (3) the ΔE is comparable to that of FdI.

3.3. EPR spectroscopy

If the three-dimensional structure of the *Anabaena* ferredoxin is a valid model for the spinach FdI, Glu-92 is not a direct ligand to the [2Fe–2S] cluster. However, its mutations are expected to have some effects on the spectroscopy of the cluster because the Glu-92 side-chain carboxylate is in contact with the [2Fe–2S] binding loop through a hydrogen bond with Ser-45. Hurley et al. [4] found no significant change in the optical spectrum of oxidized *Anabaena* ferredoxin upon the E94K mutation, and we have now confirmed this finding for the spinach FdI. Because the EPR spectrum of iron–sulfur clusters is generally much more sensitive to small structural changes, we have recorded EPR data on the reduced spinach ferredoxin ($[2Fe–2S]^{1+}$; $S = 1/2$). The results are presented in Fig. 2 (for the E92Q mutant) and Table 2. Each of the mutations of Glu-92, i.e. E92K, E92Q, E92A, affords comparable changes in the components of the g-tensor corresponding to a decrease in rhombicity. That the spectral changes are, in fact, a combination of changes in g-value and changes in line width can be seen from the derivative-shaped features in the difference spectrum, which are not centered around the peak positions of the EPR spectrum. The line width along the principal g-tensor axes is reduced indicating a decrease in g-strain.

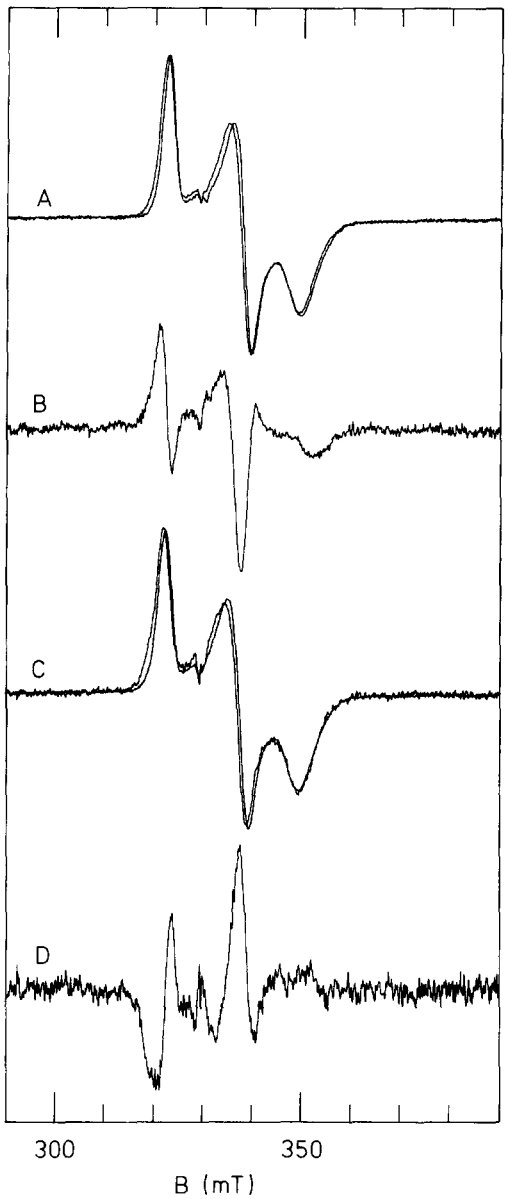

Fig. 2. Comparison of the EPR spectra of wild-type and mutant ferredoxins and of fusion protein Fd-FNR. Trace A: 240 μM wild-type and 178 μM E92Q mutant FdI; both traces are 4 × averaged and are adjusted to the same amplitude. Trace B: 3 × amplified difference of wild-type minus E92Q mutant. Trace C: wild-type FdI (as in Trace A) and 42 μM Fd-FNR; the latter is 9 × averaged. Trace D: 3 × amplified difference of wild-type FdI minus Fd-FNR. EPR conditions: microwave frequency, 9.19 GHz; microwave power, 0.2 mW; modulation frequency, 100 kHz; modulation amplitude, 0.63 mT; temperature, 20 K.

The dithionite reduced Fd-FNR spectrum also exhibits differences compared to that of wild-type FdI. These spectral changes are all opposite to those observed with the Glu-92 mutants as evidenced by the observation that the shape of the difference spectrum is the mirror image of that obtained with the E92Q mutant (cf. trace D versus trace B). There is an increase in hombicity of the g-tensor and also the line width has increased indicating an increase in g-strain.

4. Discussion

Substitution of lysine for Glu-92 in spinach FdI results in a positive shift of +93 mV in the redox potential of the [2Fe–2S]$^{(2+;1+)}$ cluster. Concomitantly, the activity of the mutant FdI with FNR in the NADPH-cytochrome *c* reductase assay increases 2.4-fold. In this assay the electron flow is measured in the direction opposite to the physiological one. The $E_m = -308$ mV of the Fd-E92K mutant is now in between the redox potentials of the reductase and of the cytochrome *c*, and this will stimulate electron transfer. In fact, there is a nearly quantitative relationship between the increase in NADPH-cytochrome *c* reductase activity and the shift in cluster redox potential for the different Glu-92 mutants. Hurley et al. [4] have reported the redox potential of *Anabaena* ferredoxin mutant E94K to be essentially unaltered. Glu-92 in spinach FdI and Glu-94 in *Anabaena* ferredoxin are fully equivalent, and therefore the mutation into lysine in both proteins would be expected to result in comparable phenotypes. A possible explanation for the observed apparent inconsistency is a difference in electrochemical methodology [15,16], although a difference in behaviour of these phylogenetically distant proteins cannot be excluded. The here presented electrochemical data on wild-type spinach ferredoxin meet the standard criteria for quasi-reversible electron transfer processes: (1) the peak-to-peak separation approaches the theoretical limit of 58/n mV for a fully diffusion-controlled process ($\Delta E = 75$ for $v = 5$ mV/s); (2) the peak current is proportional to the square-root of the potential scan rate at low values of v; (3) the ratio of cathodic over anodic peak current is close to unity; (4) the charging current, or non-Faradaic background, is 'normal', i.e. it is constant or (slightly) increasing with increasingly negative working potential. These criteria also apply to the voltammograms of the mutants E92A, E92K, E92Q, except that the quasi-reversible responses testify to an increased kinetic barrier in the inhomogeneous electron transfer as evidenced by an increased ΔE. With well developed voltammograms, as, e.g., those in Fig. 1, and when the peak separation ΔE is equal to the theoretical minimum of 58 mV ($n = 1$, $T = 20°C$), the accuracy of determining the redox potential (i.e. the average of the cathodic and anodic peak potentials) is determined by a read-out error of the order of a few mV. This number can be reduced to approximately ± 1 mV by statistics on multiple determinations. When ΔE increases due to kinetic limitations it becomes less straightforward to estimate the accuracy in E_m. In principle, it is conceivable that one peak, say the cathodic one, moves away from its 'reversible' position, while the anodic one remains in place. This would imply that, when we continue to read out E_m as the average of the peak potentials, E_m has an added uncertainty equal to $(\Delta E - 58)/2$. In practical cases this maximal error will not be reached when the increase in ΔE above 58 mV results from shifts in both the cathodic and the anodic peaks. For the cases at hand, the added error will indeed be close to zero because cathodic and anodic peaks have shifted approximately by the same amount as can be concluded from the fact that their overall shape is very similar and that $i_{p\text{-}cat}/i_{p\text{-}an}$ is close to unity. Therefore, the error in the determination of the E_m values reported in Table 1 is conservatively estimated to be of the order of ± 10 mV. Furthermore, even if the actual error would be equal to the theoretical maximum of $(\Delta E - 58)/2$, then the difference in E_m values between wild-type FdI and each of the mutants is still significant.

Table 2
EPR *g*-values of the [2Fe–2S]$^{1+}$ cluster in spinach ferredoxin mutants

FdI form	g_z	g_y	g_x
Wild-type	2.040	1.950(1)	1.882
E92K	2.037	1.946(4)	1.881
E92Q	2.038	1.947(0)	1.882
E92A	2.037	1.946(7)	1.881
Fd-FNR	2.043	1.953(7)	1.880

The *g*-values are read out directly from the spectra as peak positions (g_z and g_x) and zero crossings (g_y) and are not corrected for line-shape assymetries fron *g*-strain effects.

The response of the fusion protein is disturbed by a small amount of electrode-adsorbed FAD. This causes an added uncertainty in the determination of E_m and ΔE of the order of ± 10 mV. However, the heterogeneous electron transfer rate is still remarkably fast when compared to the rates found with the Glu-92 FdI mutants. Furthermore, the positive shift in E_m is significant even with the added uncertainty. The shift is opposite to the shift of − 22 mV determined in a spectro-electrochemical titration of wild-type spinach ferredoxin in the presence of its reductase [17]. Since the three-dimensional structure of the complex between the two proteins has not been determined it would appear premature to attempt to interpret the observed shift in atomic terms. On the other hand, the shift in E_m and the increase in ΔE are smaller than those induced by the Glu-92 mutations in FdI. Therefore, we tentatively conclude the electrochemical data to indicate that the ferredoxin part of the Fd-FNR is very similar to free FdI.

The EPR of [2Fe–2S]$^{1+}$ ferredoxins is well understood in terms of the cluster *g*-tensor [18,19] and the overall shape from *g*-strain broadening [20]. The ferric and ferrous iron are strongly coupled predominantly by Heisenberg superexchange interaction resulting in a well isolated $S = 1/2$ ground state with a cluster *g*-tensor whose low-field principal value (g_z by convention) is dominated by the contribution from the ferric ion while the other two principal values (g_y and g_x) are mainly determined by the *g*-tensor of the ferrous ion. The *g*-values reported in Table 2 are read out directly from the spectra as peak maxima (g_z and g_x) and zero crossings (g_y). These values slightly deviate from the real *g*-values that can only be determined by numerical analysis of multi-frequency data [20]. Determining these values is beyond the scope of the present work. The point to make here is that the *g*-values show a significant relative shift upon mutation of Glu-92. For all mutants, E92A, E92K, E92Q, both g_z and g_y are shifted to lower values, and this means that the coordination of both the ferric and the ferrous ion are sensitive to the mutation. For the fusion protein Fd-FNR the shifts are similar in magnitude to those in the Glu-92 mutants; however, for g_z the shift is opposite in sign (Table 2). The opposite sign simply means that the perturbation of the cluster geometry caused by the fusion is not identical to that of the Glu-92 point mutation, which is hardly a surprising observation. On the other hand, the fact that the magnitude of the perturbation is comparable to that in the mutants is remarkable, since it means that the cluster surroundings are only slightly affected indeed by the fusion with the reductase.

In conclusion, the mutation of a single glutamate at the surface of spinach FdI causes not only a change in surface charge but also structural changes within the protein resulting in a modified redox potential of the [2Fe–2S] cluster. The elim-

ination of the negative surface charge is apparent in the decrease electron transfer rate at the glassy carbon electrode; however, no such effect is detectable in the NADPH-cytochrome *c* reductase assay. On the contrary, the specific activity is increased, which indicates that not the change in surface charge but rather the change in redox potential influences the overall reaction between FNR and FdI.

Acknowledgements: We are grateful to Dr. M.A. Vanoni who was instrumental in initiating the collaboration that resulted in the present work. This investigation was supported by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST 40%), and from European Union Human Capital and Mobility, Grant ERBCHRXCT 93-0166 to the FLAPS network.

References

[1] Zanetti, G., Morelli, D., Ronchi, S., Negri, A., Aliverti, A. and Curti, B. (1988) Biochemistry 27, 3753–3759.
[2] Correl, C.C., Ludwig, M.L., Bruns, C.M. and Karplus, P.A. (1993) Protein Sci. 2, 2112–2133.
[3] De Pascalis, A.R., Jelesarov, I., Ackermann, F., Koppenol, W.H., Hirasawa, M., Knaff, D.B. and Bossard, H.R. (1993) Protein Sci. 2, 1126–1135.
[4] Hurley, J.K., Salamon, Z., Meyer, T.E., Fitch, J.C., Cusanovich, M.A., Markley,J.L., Cheng, H., Xia, B., Chae, Y.K., Medina, M., Gomez-Moreno, C. and Tollin, G. (1993) Biochemistry 32, 9346–9354.
[5] Rypniewski, W.R., Breitner, D.R., Benning, M.W., Wesenberg, G., On, B.-H., Markley, J.L., Rayment, I. and Holden, H.M. (1991) Biochemistry 30, 4126–4131.
[6] Holden, H.M., Jacobson, B.L., Hurley, J.K., Tollin, G., Oh, B.-H., Skjeldal, L., Kee Chae, Y., Cheng, H., Xia, B. and Markley, J.L. (1994) J. Bioenerg. Biomembr. 26, 67–88.
[7] Aliverti, A., Corrado, M.E. and Zanetti, G. (1994) FEBS Lett. 343, 247–250.
[8] Piubelli, L., Aliverti, A., Bellintani, F. and Zanetti, G. (1995) Protein Express. Purif. 3, (in press).
[9] Hagen, W.R. (1989) Eur. J. Biochem. 182, 523–530.
[10] Verhagen, M.F.J.M., Link, T.A. and Hagen, W.R. (1995) FEBS Lett. 361, 75–78.
[11] Pierik, A.J. and Hagen, W.R. (1991) Eur. J. Biochem. 195, 505–516.
[12] Nicholson, R.S. (1965) Anal. Chem. 37, 1351–1355.
[13] Nicholson, R.S. and Shane, I. (1964) Anal. Chem. 36, 706–723.
[14] Bagby, S., Baker, P.D., Hill, H.A.O., Sanghera, G.S., Dunbar, B., Ashby, G.A., Eady, R.R. and Thorneley, R.N.F. (1991) Biochem. J. 277, 313–319.
[15] Salamon, Z. and Tollin, G. (1991) Bioeletrochem. Bioenerg. 26 (J. Electroanal. Chem. 321), 321–334.
[16] Salamon, Z. and Tollin, G. (1992) Bioelectrochem. Bioenerg. 27 (J. Electroanal. Chem. 342), 381–392.
[17] Smith, M., Smith, W.H. and Knaff, D.B. (1981) Biochim. Biophys. Acta 635, 405–411.
[18] Gibson, J.F., Hall, D.O., Thornley, J.H.M. and Whatley, F.R. (1966) Proc. Natl. Acad. Sci. USA 56, 987–990.
[19] Bertrand, P. and Gayda, J.-P. (1979) Biochim. Biophys. Acta 579, 107–121.
[20] Hearshen, D.O., Hagen, W.R., Rands, R.H., Grande, H.J., Crespi, H.L., Gunsalus, I.C. and Dunham, W.R. (1986) J. Magn. Reson. 69, 440–459.